DEPARTMENT OF HEALTH & HUMAN SERVICES

PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH

FOR US POSTAL SERVICE DELIVERY:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500, MSC 6910
Bethesda, Maryland 20892-6910
Home Page: http://grants.nih.gov/grants/olaw/olaw.htm

FOR EXPRESS MAIL:
Office of Laboratory Animal Welfare
6700B Rockledge Drive, Suite 2500
Bethesda, Maryland 20817
Telephone: (301) 496-7163
Facsimile: (301) 480-3387

December 16, 2020

Re: Animal Welfare Assurance
A4051-01 [OLAW Case X]

Dr. Wayne E. Cascio
Deputy Director, National Health and
Environment Effects Research Laboratory
U.S. Environmental Protection Agency
EPA-MD-B305-01, 109 T.W. Alexander Drive
Research Triangle Park, NC 27709

Dear Dr. Cascio,

The Office of Laboratory Animal Welfare (OLAW) acknowledges receipt of your December 3, 2020 letter reporting an instance of noncompliance with the PHS Policy on Humane Care and Use of Laboratory Animals at the United States Environmental Protection Agency (US EPA) following up on an initial November 18, 2020 notification by email.

According to the information provided, this Office understands that the US EPA Animal Care and Use Committee (ACUC) determined that instances of noncompliance occurred with respect to: the deaths of 118 fish fry on a rack in the fish facility. The final report states the affected fish were being reared for future breeding and were the only population of animals on the rack. HVAC system renovations have been underway all year for all campus buildings. Animal care staff were notified by construction staff that temperatures during HVAC construction would not increase but instead decrease.

On Friday, November 13, 2020 a remote alarm system on a fish rack sent out a maximum temperature alarm notification. The report states this rack is in a different room where the mortality would later occur. Due to cold weather, the heat activated throughout campus and room temperatures in the fish suite increased. The facility manager (FM) arrived on site and along with the on-site facilities contractor deployed portable air-conditioning units to decrease the temperatures. An additional unit was set up on November 14th in the afternoon in the third fish holding room, where the fish mortality would occur 4 days later. Temperatures in the fish rooms were well above the 75°F set point for the spaces and tanks reached 31°C (normal temp. is 28°C). Room air temperatures decreased to an acceptable range on Sunday and tanks were back within normal range as well.

On Monday, November 16th, daily rack records indicated the pH was 7.4 for the rack that would experience fish mortality on Wednesday. The pH probe was recalibrated, and subsequent pH reading was 8.13, which is above the 7.4 set point. It is stated verbal reports indicated a second method of pH reading may have been conducted (as required by protocol). However, there are no records of a second reading, and no documentation of any further actions or contacting the FM, AV or anyone else about the elevated pH reading as required by facility operating procedure. The pH readings for Tuesday could not be determined because the records have been crossed out and written over, and therefore difficult to discern. The AS was on site and initiated steps to decrease the pH to within acceptable range, which included increasing the rate of water exchange for the rack.

On Wednesday, November 18th, the zebrafish fry were found dead. The sump float valve on the rack was stuck open, and the dosing tank for salt solution was found depleted. The pH readings returned to normal, but the conductivity reading was half that of usual, likely due to the continuous water exchange. The veterinarian and fish experts both within and outside of EPA reviewed the incident and determined if the pH did indeed stay at 8 or so for any length of time, that pH alone could have been responsible for fry mortality. The reviewers agreed that all insults delivered to the fry in rapid succession would certainly kill the high percentage of young fish. The program is initiating the following actions in response to this event:

- All fish/fry have been removed from the affected rack to a more modern system which currently has a more stable water supply.

- The portable air-conditioning units have been left in place in each room that has a fish population, plugged in, and the thermostat set so the units come on when room temperatures elevate outside of the accepted range (above 73°F). These units will remain in place until HVAC renovations are complete and the building room air temperature monitoring system comes back on-line for this area.

- The Animal Resources Program Office (ARPO) is looking into purchase of water chillers for the fish housing systems

- ARPO is looking into the failure of the sump float on the affected rack.

- ARPO is considering looking into replacing the older stand-alone fish racks with more modern ones that come with alarm systems, built in water exchange units, and built in heaters and chillers in addition to the usual aquatic life support requirements.

- Program management has re-emphasized that, per facility operating procedures, water quality readings, like pH, outside the normal range need to be confirmed and documented, and once confirmed, the readings need to be immediately reported to the FM, AS and AV or alternate designees so an appropriate course of action can be determined.

- The IACUC is requiring retraining for staff on water quality, water testing, and reporting methods and protocols including communication of adverse conditions. The IACUC will be updated as this training proceeds.

Based on its assessment of this explanation, OLAW understands that the US EPA has implemented appropriate measures to correct and prevent recurrences of these problems and is now compliant with provisions of the PHS Policy. We appreciate being informed of these matters and find no cause for further action by this Office.

Sincerely,

Jacquelyn T. Tubbs -S
Digitally signed by Jacquelyn T. Tubbs -S
Date: 2020.12.16 15:15:50 -05'00'

Jacquelyn T. Tubbs, DVM
Animal Welfare Program Specialist
Division of Compliance Oversight
Office of Laboratory Animal Welfare

cc: IACUC Contact

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CENTER FOR PUBLIC HEALTH AND ENVIRONMENTAL ASSESSMENT
Research Triangle Park, NC 27711

OFFICE OF
RESEARCH AND DEVELOPMENT

December 3, 2020

Brent C. Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

Dear Dr. Morse;

The purpose of this letter is to report an animal welfare issue at the US EPA Research Triangle Park vivarium (Assurance # D16-00569, (b) (6) and to report on the measures being taken by the program to prevent similar events from occuring in the future. A preliminary report of this event was originally emailed to OLAW by our IACUC Chair on Wednesday, November 18, 2020.

On the morning of 11/18/2020, 118 fish fry out of a population of about 450 were found dead on one rack in one room of the fish facility; a death of greater than 25% of the population for that rack. This triggered an investigation into this event. The affected fish were juvenile zebrafish fry which were being reared for future breeding. The fish had not been treated or exposed to anything other than standard rack water and were the only population of animals on this rack.

Renovations of the HVAC system of all campus buildings have been proceeding all year. Renovations to the floor housing the fish facility began October 26, 2020. Construction staff assured animal care staff and researchers that temperatures during the HVAC construction would drop, not rise.

On Friday, 11/13/20 at 11:51 PM a fish rack equipped with a remote alarm system, located in a different room from the rack where there later was fish mortality, sent out a maximum temperature alarm notification. Room and water temperatures had been within normal ranges earlier that Friday during regular work hours. That Friday night was one of the first cold nights of the year and the heat had kicked on throughout campus. Room temperatures in the fish suite rose.

The facility manager (FM) arrived on site to attend to the alarm at approximately 12:40 AM and began working with the fish housing systems to reduce temperatures. Protective tents that had been placed to protect the fish tanks from potential debris from the HVAC renovations were rolled away. The FM worked with the on-site facilities contractor to deploy portable air-conditioning units to bring the temperatures down. Two air conditioning units were set up shortly before 1 AM Saturday morning 11/14/20 in two of the fish holding rooms and ran until 3 AM,

then were re-deployed around 7:45 AM by the Aquatics Specialist (AS). An additional unit was set up some time Saturday afternoon in the third fish holding room, the room holding the rack that would experience fish mortality 4 days later. A barn fan was deployed in the central area of the suite, moving the heated air out of the rooms into the service corridor. Temperatures in the fish rooms were in the 80's, well above the 75F set point for the spaces and tanks had reached 31C (normal temperature is 28 C). Room air temperatures had dropped to 72-73F by Sunday, back to an acceptable range, and tanks were back down on average to 28C.

On Monday, 11/16 the morning daily rack records indicate the pH was 7.4 for the rack that would experience mortality on Wed 11/18. The pH probe on this rack was recalibrated sometime in the afternoon. The subsequent reading was high – pH 8.13, well above the 7.4 set point. The elevated reading was attributed to the recalibration of the probe, as elevated readings are normal until the re-calibrated probe settles. While verbal reports indicate a second method of reading the pH may have been used as required by protocol, there are no records of this second reading, and no documentation of any further actions or of contacting the FM, AV or anyone else about the elevated pH readings as required by facility operating procedure. The pH of this rack water may have been 7.8 on Tuesday morning, but the records have been crossed out and written over, so it is difficult to read. The AS was on site in the facility and took steps to drop the pH back to acceptable range, including increasing the rate of water exchange for this rack.

On Wednesday morning 11/18/20, the zebrafish fry were found dead. The sump float valve that keeps the sump water level constant on the affected rack was found stuck, and the dosing tank for salt solution was found depleted. Water exchange appears to have happened continuously overnight. The pH for the rack was back down to normal ranges, but the conductivity reading was about half that of usual, likely due to the continuous water exchange.

The attending veterinarian and fish experts both within and outside EPA reviewed the situation and consider if the pH did indeed stay at 8 or so for any length of time, that pH alone could have been responsible for fry mortality. Zebrafish fry are more sensitive to pH than adult fish. If, as staff suggest, this was an aberrant pH reading solely due to the recalibration of the probe, the other insults - high temperature, low conductivity, and excessive water exchange - could have combined to lethal effect. The reviewers agreed that all insults delivered to the fry in rapid succession would certainly kill the high percentage of young fish.

The program is working to ameliorate current and future problems as follows:

1. All fish/fry have been removed from the affected rack to a more modern system which currently has a more stable water supply.
2. The portable air-conditioning units have been left in place in each room that has a fish population, plugged in, and the thermostat set so the units come on when room temperatures elevate outside of the accepted range (above 73F). These units will remain in place until the HVAC renovations are complete and the building room air temperature monitoring system comes back on-line for this area.
3. The Animal Resources Program Office (ARPO) is looking into purchase of water chillers for the fish housing systems. ARPO will be consulting with other aquatic facilities about the advisability of using chillers in tropical systems.
4. ARPO is looking into the failure of the sump float on the affected rack.

5. ARPO is considering looking into replacing the older stand-alone fish racks with more modern ones that come with alarm systems, built in water exchange units, and built in heaters and chillers in addition to the usual aquatic life support requirements (pH probes, conductivity probes, UV systems, filtration systems). The rack housing the fish that died is an older piece of equipment.
6. Program management has re-emphasized that, per facility operating procedures, water quality readings, like pH, outside the normal range need to be confirmed and documented, and once confirmed, the readings need to be immediately reported to the FM, AS and AV or alternate designees so an appropriate course of action can be determined.
7. The IACUC is requiring retraining for staff on water quality, water testing, and reporting methods and protocols including communication of adverse conditions. The IACUC will be updated as this training proceeds.

We hope these measures satisfactorily address the issues. Please contact Steve Gavett (b) (6) (b) (6) Gavett.stephen@epa.gov), the IACUC Chair, or Jaimie Graff (b) (6) Graff.jaimie@epa.gov), Animal Resources Program Office, if you have further questions.

Sincerely,

Wayne E. Cascio, M.D.
Director, CPHEA
Institutional Official

Stephen H. Gavett

Stephen H. Gavett, Ph.D., D.A.B.T.
CPHEA / PHITD / CIB
IACUC Chair

Wolff, Axel (NIH/OD) [E]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, December 9, 2020 10:11 AM
To: Gavett, Stephen
Cc: OLAW Division of Compliance Oversight (NIH/OD)
Subject: RE: Final report of adverse animal welfare incident and corrective actions

Thank you for this report, Dr. Gavett. We will send a response soon.

Axel Wolff, M.S., D.V.M.
Deputy Director, OLAW

From: Gavett, Stephen <Gavett.Stephen@epa.gov>
Sent: Wednesday, December 9, 2020 10:06 AM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; (b) (6) (b) (6) Jarrell, Leslie <Jarrell.Leslie@epa.gov>
Subject: Final report of adverse animal welfare incident and corrective actions

Dear Dr. Morse:

We have completed the investigation of the zebrafish fry deaths which occurred at our institution (US EPA, Office of Research and Development, Research Triangle Park Program; OLAW Assurance number D16-00569, A4051-01), as initially reported below on 11/18/2020.

A draft report of the incident and proposed corrective actions was discussed at the meeting of our IACUC on 12/2/2020. The IACUC approved the report, agreeing that it accurately described the conditions which led to the deaths and provided appropriate and necessary corrective actions to avoid future incidents. The attached final report from our Institutional Official Dr. Wayne Cascio provides the details of the adverse conditions and the corrective actions which are being implemented.

Please let us know if you need any further information. We look forward to your response.

Sincerely,

Stephen Gavett, Ph.D., D.A.B.T.

Research Biologist
Cardiopulmonary and Immunotoxicology Branch
CIB PHITD CPHEA ORD USEPA
Tel. (b) (6)
Mailing Address:
U.S. Environmental Protection Agency
Attn: Stephen Gavett
Mail Code: B105-02
Research Triangle Park, NC 27711

From: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Sent: Wednesday, November 18, 2020 4:59 PM
To: Gavett, Stephen <Gavett.Stephen@epa.gov>

Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; (b) (6) ; Jarrell, Leslie <Jarrell.Leslie@epa.gov>; OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Preliminary notice of animal welfare issue

Thank you for this prompt preliminary report Dr. Gavett. We will open a case file and await further information as noted in your email.

Best regards, Brent Morse

Brent C. Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: Gavett, Stephen <Gavett.Stephen@epa.gov>
Sent: Wednesday, November 18, 2020 4:32 PM
To: OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; (b) (6) ; Jarrell, Leslie <Jarrell.Leslie@epa.gov>
Subject: Preliminary notice of animal welfare issue

To the Division of Compliance Oversight:

As chair of our IACUC, I am writing to provide preliminary notice of an adverse event impacting animal welfare which was identified today, Wednesday November 18, 2020, at our institution (US EPA, Office of Research and Development, Research Triangle Park Program; OLAW Assurance number D16-00569, A4051-01).

A rack of zebrafish tanks housing fry being reared for future breeding was found with approximately 25% of the population dead this morning. Initial findings suggest elevated temperature over the weekend due to ongoing renovations combined with altered water quality and excessive water exchange from a stuck float yesterday may have contributed to these deaths. We are currently undertaking a thorough review of events leading to these deaths. A final report from our Institutional Official (Dr. Cascio) with corrective actions will be delivered to OLAW as soon as the facts are gathered.

Please let me know if you need further information.

Sincerely,

Stephen Gavett, Ph.D., D.A.B.T.

Research Biologist
Cardiopulmonary and Immunotoxicology Branch
CIB PHITD CPHEA ORD USEPA
Tel. (b) (6)
Mailing Address:
U.S. Environmental Protection Agency
Attn: Stephen Gavett
Mail Code: B105-02
Research Triangle Park, NC 27711

A4051 – X

Walker, Keri (NIH/OD) [C]

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, November 18, 2020 5:00 PM
To: Walker, Keri (NIH/OD) [C]
Subject: FW: Preliminary notice of animal welfare issue A4051

Follow Up Flag: Follow up
Flag Status: Flagged

Keri,

Please use this email to open a new case file. Assign it to me. Thank you. Brent

Brent C. Morse, DVM, DACLAM
Director, Division of Compliance Oversight
Office of Laboratory Animal Welfare
National Institutes of Health

From: OLAW Division of Compliance Oversight (NIH/OD)
Sent: Wednesday, November 18, 2020 4:59 PM
To: Gavett, Stephen <Gavett.Stephen@epa.gov>
Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; (b) (6) (b) (6) ; Jarrell, Leslie <Jarrell.Leslie@epa.gov>; OLAW Division of Compliance Oversight (NIH/OD) <olawdco@od.nih.gov>
Subject: RE: Preliminary notice of animal welfare issue

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Best regards, Brent Morse

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Office of Laboratory Animal Welfare
National Institutes of Health

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Cc: Cascio, Wayne <Cascio.Wayne@epa.gov>; Graff, Jaimie <Graff.Jaimie@epa.gov>; (b) (6) (b) (6) ; Jarrell, Leslie <Jarrell.Leslie@epa.gov>
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A rack of zebrafish tanks housing fry being reared for future breeding was found with approximately 25% of the population dead this morning. Initial findings suggest elevated temperature over the weekend due to ongoing renovations combined with altered water quality and excessive water exchange from a stuck float yesterday may have contributed to these deaths. We are currently undertaking a thorough review of events leading to these deaths. A final report from our Institutional Official (Dr. Cascio) with corrective actions will be delivered to OLAW as soon as the facts are gathered.

Please let me know if you need further information.

Sincerely,

Stephen Gavett, Ph.D., D.A.B.T.

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